**Identification of plant viruses-**

The aim of identification of viruse is to –

1. Determination of shape and size
2. Determination of physical properties of virus
3. Determination of host range
4. Determination of insects vectors
5. Determination of virus identity by serological method
6. **Determination of Size and Shape**

The size and shape of a virus is determined by electron microscopy. A purified or semi-purified virus preparation is normally used. This requires several cycles of high and low speed centrifugation and subsequent density centrifugation.

Examination from crude sap is also possible, and is described here:

**Materials needed:**

1. Copper grids

Size depends on the electron microscope used. Grids of 3 mm diameter and 150-400 mesh size are the most common. (The mesh size indicates the number of apertures per grid.)

The grids must be coated so that they can support the virus particles. Coating grids requires skill and practice, though, and it is often easier to obtain precoated grids from a virologist at a cooperating institute.

**2. The following materials can be used for coating:**

- **Collodion 0.2% in amylacetate**. This support film is easy to prepare, but is relatively unstable.

- **Formvar 0.2% in 0.5% chloroform or ethylenedichloride**. Although slightly more difficult to prepare, this support film has the advantage of being more stable. It can be **stabilized** even further by adding **carbon**, but this can only be done with a special apparatus found in the **virology departments of universities and research institutes**.

- Fine pointed stainless steel forceps (for manipulating the grids)

- Grid box (for storage and transport of the grids)

- Dust-free double-distilled water

 **3. Chemicals:**

**Stains:**

 Uranylacetate (UAC)

 Phosphotungstic acid (PTA)

Grid coating chemicals:

 Formvar (Polyvinyl formaldehyde)

 Collodion (Parlodion)

**Electron Microscopic Examination**

* Observe the specimen at approximately 32,000 X magnification.
* Photograph it at 5,000 X magnification. (Higher magnification can be achieved with photographic enlargement.)
* Contaminating plant particles such as chloroplast fragments and ribosomes may make it difficult to distinguish virus particles. Clear preparations

**2. Determination of the Physical Properties of the Virus**

**(2a) Thermal Inactivation Point (TIP)**

Definition: The TIP is the temperature required to completely inactivate the virus in crude sap during a ten minute exposure. The thermal inactivation point (TIP) is defined as the lowest temperature required for complete inactivation of a virus in crude sap heated for 10 min. In the case of an unidentified virus, virus-containing sap is first exposed to temperatures at 10 °C intervals. When repeating the test, the intervals are usually narrowed down to 5 °C in the range of inactivation. For example, if the inactivation occurred between 40 and 50 °C, repeat the test between 35 and 55 °C at 5 °C intervals. In view of the great variation occurring in this type of tests due to the aforementioned factors, it makes no sense to heat at intervals smaller than 5 °C. In the literature, TIP is usually reported as the two temperatures in between which the virus is inactivated completely. Viruses with elongate particles have a high temperature coefficient (Q10°; the ratio of velocity constants of inactivation at temperatures differing by 10 °C) and their inactivation is accompanied by denaturation of their coat protein. Viruses with isometric particles, on the other hand, have a low Q10°, and their inactivation takes place long before denaturation of their coat protein. With this type of viruses, unlike the former, temperature is not the most important factor determining inactivation and, therefore, there is a great variation in TIPs reported in the literature.

Method: Homogenize the infected leaf tissue with small amount of buffer. Pass the crude sap through cheesecloth. With a pipette add 2 ml of the sap to each of eight screw-capped test tubes, being careful not to let the sap drip along the walls of the test tube. Each tube is heated in a water bath for ten minutes. Preliminary testing should be at 10°C intervals (30°C to 100° C).

After heating, the tubes are cooled immediately in Ice cold water.

Test plants, preferably those which will react to the virus with local lesion formation, are then inoculated with the samples. The test plants are observed for symptoms for four days to three weeks, and the temperature range in which virus activity ceases is recorded. For determination of the exact TIP, this temperature range is then divided into five smaller Intervals (e.g. 59, 62, 65, 68, and 71 °C). Five test tubes with sap prepared in the same manner as described above are heated, and test plants are Inoculated. The lowest temperature at which no symptoms appear on the inoculated test plants is the TIP.

**(2b) Longevity In-Vitro (LIV)**

Definition: The LIV is defined as the length of time the virus is infective in crude sap kept at room temperature (approximately 20 to 22°C). Longevity in vitro (LIV) is the length of time after which crude sap from a virus-infected plant loses its infectivity when kept at room temperature (20–22 °C). To determine the LIV of a virus, samples of crude sap are removed from storage at intervals and tested on assay plants. In the absence of information on the stability of a virus in sap, the first series of intervals should be at a geometric progression, e.g. 1, 2, 4, 8, 16, 32...days, until infectivity is lost. As soon as the LIV has been roughly established, the test can be repeated over a narrower range of shorter intervals.

Method: Use a clarified extract similar to that used to test for TIP, but with 0.01% Streptomycin or Aureomycin added. (These are antibiotics which prevent bacterial contamination.) Ten screw-capped test tubes are each filled with 2 ml of sap. Test plants (preferably local lesion hosts) are inoculated at various time intervals (1, 3, 6, 9, 12, 15, 30, 60, 90, 150 days) and observed for symptom development. If symptoms appear at the 60 day interval but not at the 90 day interval, the LIV is between 60 and 90 days. For an exact LIV determination, time intervals of 2 to 5 days should then be tested within the range of 60 to 90 days.

**(2c) Dilution End Point (DEP)**

Definition: The DEP is the highest dilution of plant sap in which a virus is still infectious.

Method: Homogenize infected leaf tissue in a small amount of buffer. Several dilutions can then be made from this undiluted sap: 10-1, 10- 2, 10-3 , 10- 4 , 10- 5 , 10-6, 10-7 and 10-8.

10-1 dilution: I ml undiluted sap + 9 ml buffer (Shake well.)

10 -2 dilution: I ml of 10-1 dilution + 9 ml buffer (Shake well.)

 10-3 dilution: 1 ml of 10-2 dilution + 9 ml buffer (Shake well.)

 Use a similar procedure to make additional dilutions.

Inoculate in host plant and record the highest dilution which still produces symptoms on the inoculated plants.

**3. Determination of Host Range**

Grind one part infected plant tissue with five parts buffer, squeeze it through a cheesecloth, and inoculate in various test plants followed by mechanical method of sap inoculation and record for symptoms to occur.

**D. Determination of Insect Vectors**

When mechanical transmission fails, place insects on infected plants for acquisition feeding. After feeding, place the insects on healthy plants for transmission feeding, and observe the plants for symptom development.

**E. Determination of Virus Identity by Serological Methods**

Most serological methods are based on the precipitation produced when antibodies (the aniserum) and antigens (the virus) combine. Antisera must be prepared from purified or semi-purified virus preparations.

Many antisera can also be ordered from:

ATCC (American Type Culture Collection) 12301 Parklawn Drive Rockville Maryland 20852 USA

The serological tests most commonly used are:

- Microprecipition test in thin, small test tubes

 - Ouchterlony agar gel double diffusion test in petri dishes

- Immunosorbent electron microscopy (ISEM)

 - Enzyme-linked immunosorbent assay (ELISA)

These tests are applicable to virus Identification from crude sap, clarified sap, and purified preparations. They require special training, and, in the case of ISEM and ELISA, special equipment which is not commonly available.